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# AN *IN VITRO* METHOD FOR THE SCREENING OF TRICHOTHECENE MYCOTOXIN CYTOTOXICITY AND THE STUDY OF MYCOTOXIN COUNTERMEASURES (U)

by

V.L. DiNinno, D. Penman, A.R. Bhatti,
N.P. Erhardt and P.A. Lockwood

ACN No. 16A10

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## ABSTRACT

A thymocytic cell bio-assay was developed for assessing the toxicity of the trichothecene mycotoxin T-2. The *in vitro* toxicity of mycotoxin T-2 towards mouse mono-nuclear leukocytes prepared from spleen, thymus, peritoneal lavage and bone marrow cells was studied. Bone marrow cells were found to be more resistant to T-2 toxicity than thymus, spleen and peritoneal cell preparations. This method is rapid and provides consistant and reproducible results for the study of T-2 mycotoxicosis.

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## INTRODUCTION

Several papers have described the action of trichothecene mycotoxins on prokaryotic cellular systems (1 – 4). This information, however, may not be directly relevant to mammalian systems in terms of mycotoxin toxicity and mode of action. Considerable work has been done on the feeding of mammalian species with trichothecene mycotoxins (5 – 8) and while these have the advantage of being more directly related to mammalian eukaryotic cells and thus, inferentially, to human cytopathogenicity, the methods used are slow (4 – 6 weeks) and time consuming.

While the histological cellular evidence of such orally administered mycotoxins is well documented (9), this method lacks precision with respect to each quantitative challenge posed to cells sensitive to the effects of the mycotoxin. Lafarge-Frayssinet *et al.* (9), have demonstrated the effects of exposing certain cellular elements of the reticuloendothelial system in test mammals to various dosage challenge of mycotoxins.

It is felt that the work of Lafarge-Frayssinet *et al.* (9), could be developed to provide a rapid (7 – 22 hrs) *in vitro* method for the presumptive screening of the cytotoxic actions of mycotoxins and that this cytotoxicity could be related to the action of certain mycotoxins and/or their secondary metabolites in animals. In turn, this could also bear relationship to certain specific molecular configurations of these mycotoxin molecules.

In this paper, the development of an *in vitro* system using murine thymocytic cells as a bio-assay for a trichothecene mycotoxin is described. The method is rapid and has provided consistent and reproducible results for the study of T-2.

## MATERIALS AND METHODS

### Cells and Culture Conditions

Cells were maintained for the duration of the experiment in Eagle's modified minimal essential media with glutamine (Flow Laboratories) containing 2 mL of 1 M HEPES buffer and 1 mL (5000 units of penicillin, 500 μg streptomycin) antibiotic solution per 100 mL of media. The cells were cultured in 12 × 75 mm polystyrene disposable culture tubes in an incubator at 37°C in a 5% $C0_2$ atmosphere.

### Preparation of Mouse Cells Suspensions

C-57 black male mice, 3 – 6 weeks of age, were used for all experiments. Mice were sacrificed by $C0_2$ inhalation and flooded with 70% ethanol prior to dissection. Spleen cell, thymocytes, bone marrow cells and peritoneal cells were prepared as described by Mishell and Shiigi (10).

**Viability Assay**

Mycotoxin T-2 (Sigma Chemical Co.) was dissolved (20 mg/mL) in dimethylsulphoxide (DMSO). Toxin dissolved in DMSO was added to 500 μL of cells adjusted to $2 \times 10^7$ cells/mL. The amount of DMSO added to cell cultures did not exceed 25 mL. Appropriate controls were also set up and the viability of the cell suspension was assessed at hourly intervals by removing an aliquot and mixing it with an equal volume of 0.2% Eosin Y solution prepared with culture medium. A percentage viability count was determined by counting in a Neubauer hemocytometer; four areas containing a total of not less than 400 cells were counted in each instance. All statistical analyses were performed using a statistical package for F test analysis and linear regression, available for the Tektronix 4054 (Tetronix Inc., Beaverton, Oregon).

**RESULTS AND DISCUSSION**

Trichothecene mycotoxin T-2 produces widespread degenerative and necrotic changes in lymphoid cells of spleen, thymus, lymph node and bone marrow. In spite of this toxicity, there is regeneration and repopulation of lymphoid tissue even upon prolonged exposure to T-2 toxin in feed (8). This repopulation phenomona indicates either the existence of some hemopoetic cell line resistant to T-2 mycotoxin and therefore capable of repopulating the various lymphoid organs or the slow adaptation of hemopoetic cells to the toxic effects of T-2 mycotoxin. Figure 1 illustrates the percent survival of various lymphoid cells upon exposure to a standard dose of 400 μg of T-2 mycotoxin per $2 \times 10^7$ cells/mL. Thymocytes (Figure 1A) and peritoneal lavage cells (Figure 1B) are exquistely sensitive to the effects of T-2 mycotoxin, showing virtually no survival after 5 – 6 hr exposure. Spleen cells (Figure 1D) show an intermediate pattern of survival, and while less sensitive than thymocytes, still show a rapid decline in viability. Bone marrow cells (Figure 1C), on the other hand, proved to be quite resistant to T-2 cytotoxicity showing only a small but significant decline in viability. A large proportion of the heterogenous population of bone marrow cells are resistant to T-2 mycotoxin and, even if incubated for 24 hr (data not included) in the presence of the mycotoxin, showed a 40 – 50% survival. While this resistant population awaits identification, it is suggested that these may be the cells which allow the lymphoid organs to be repopulated even upon prolonged exposure to T-2 mycotoxin.

In utilizing such a system for an *in vitro* screening of trichothecene toxicity, the use of thymocytes is proposed. Figure 1, clearly shows the advantage of using thymocytes over bone marrow and/or spleen cells. Thymocytes are also preferred over peritoneal cells, because of the higher yield of thymocyte cells available from a mouse.

The use of thymocytes for the purpose of detection would not circumvent the lack of specificity associated with other biological methods (11), but it is, nevertheless, a rapid and simple technique. More importantly, however, it offers an *in vitro* method to study the relationship between molecular structure and trichothecene toxicity. In addition, this system lends itself to the screening and study of chemical countermeasures against the deleterious cytotoxic effects of mycotoxins.

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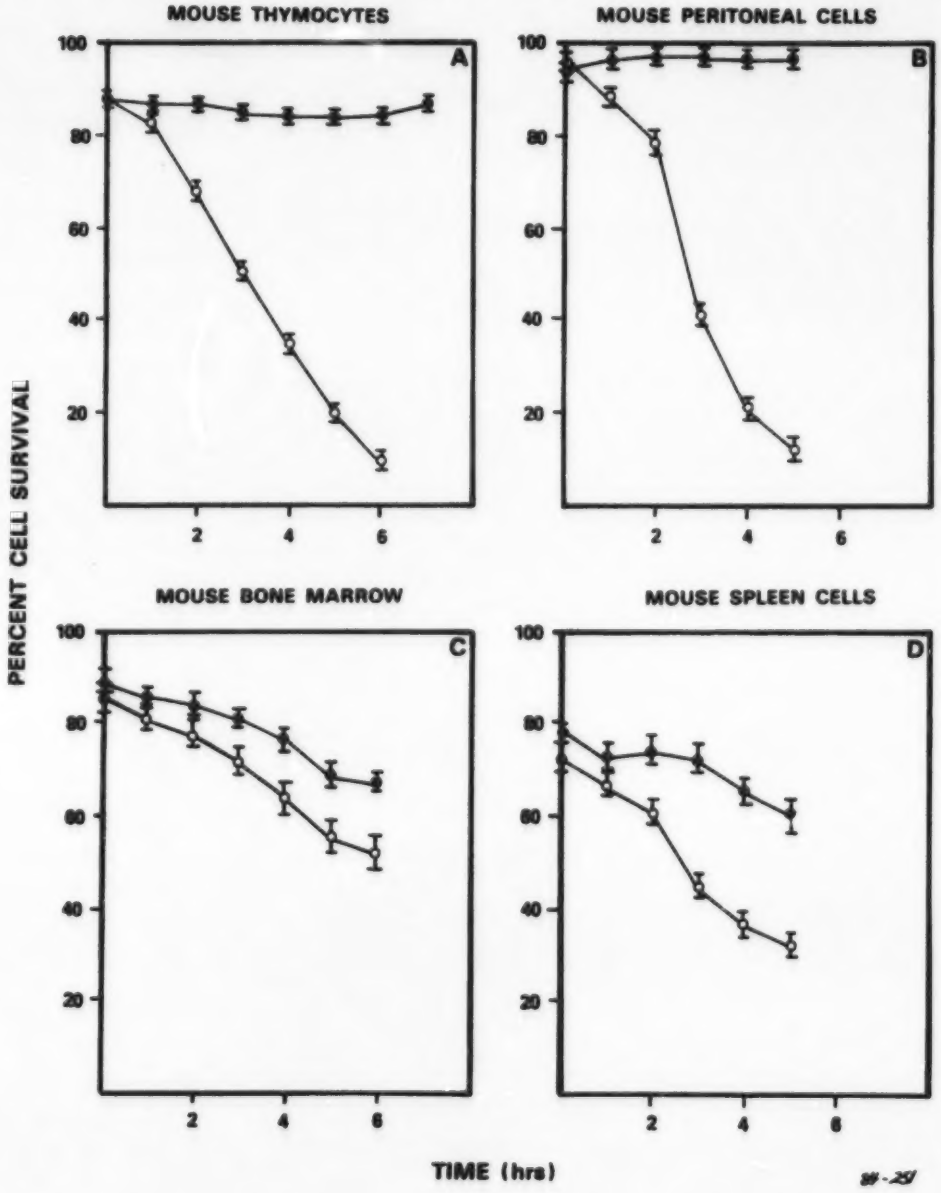


**Figure 1**

**The % survival of mouse thymocytes (A), peritoneal lavage (B), bone marrow (C) and spleen cells (D). In each case, $2 \times 10^7$ cells were exposed to 400 μg of T-2 toxin and the % survival calculated at hourly intervals. The mean and standard error of not less than five separate experiments are presented. Solid and hollow circles represent control and T-2 treated cells, respectively.**

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